Bangladesh J. Bot. **34**(1): 1-6, 2005 (June)

DETERMINATION OF POLLEN QUALITY AND QUANTITY IN CORNELIAN CHERRY (*CORNUS MASS* L.)

LÜTFI PIRLAK AND MUHARREM GÜLERYÜZ[1]

Ataturk University, Faculty of Agriculture, Department of Horticulture, Erzurum/Turkey

Key words: Pollen quality and quantity, Cornelian Cherry, *Cornus mass*, Turkey

Abstract

The pollen grains of five Cornelian cherry types (25-Uz-34, 25-Uz-39, 25-Uz-43, 25-Uz-53 and 25-Uz-61) in Uzundere district of Erzurum, Turkey were tested for the determination of viability, germination rate, pollen production level and morphological homogeneity. Viability of the pollens was determined by Triphenyl tetrazolium chloride and Iodine-potassium iodide tests. Pollen germination tests were carried out with 'Hanging Drop' method in sucrose solutions of 0, 5, 10, 15, 20 and 25% and in 0.03, 0.05, 0.1 and 0.2% boric acid. In addition, pollen production and morphological homogeneity were determined by the 'Hemacytometric method'. Pollen germination rates were highest in 15 and 20% sucrose and 0.03% boric acid solutions. The maximum pollen production level was obtained from the 25-Uz-53 variety. The morphological homogeneity level of pollens ranged from 92.39 to 95.96%.

Introduction

Cornelian cherry (*Cornus mas* L.) is a commonly grown fruit plant in Turkey. Turkey has different Cornelian cherry types and forms because Turkey is one of the country of origin of Cornelian Cherry. Most of the trees are feral, but some are under cultivation. Cornelian cherries are mainly grown in the Aegean, Mediterranean, Black Sea and North eastern Anatolia regions of Turkey (Güleryüz and Pirlark 1996). Cornelian cherry production in Turkey is about 12800 t/year (Anon 2001).

The main aim of fruit growing plants is to increase yields with distinct quality and greater quantity. As similar to other fruit species, it is important for Cornelian cherry to improve its yield and quality. For this purpose, it is also important to understand the problem in fertilization biology as well as technical problems. Information on fertilization biology of Cornelian cherry is limited. The viability, germination rate and morphological homogeneity related to pollen quality are the most important properties in fruit trees. These properties are useful for plant breeders and growers. Low fertilization rate and fruit set are closely related to different properties of pollen characteristics (quantity, germination rate and morphological homogeneity). Identifying the relations between fertilization and these properties are very important for practical fruit growing (Stösser 1984).

Fertilization biology for any fruit species is only possible with studies under orchard conditions, since real fruit yields could be ascertained under the effects of local ecological conditions and compatibility. However, studies on pollen germination and viability under laboratory conditions produce valuable information about fruit species (Eti 1991).

The viability, germination and production of pollen are not known exactly in Cornelian cherry and thus the present study was to investigate the above problems.

---

[1]Selcuk University, Faculty of Agriculture, Department of Horticulture, Seleuklu, Kampus Konya/Turkey.

Materials and Methods

Five Cornelian cherry types (25-Uz-34, 25-Uz-39, 25-Uz-43, 25-Uz-53, 25-Uz-61) previously selected from Uzundere-Erzurum (Pirlak 1993) were used as source materials. The balloon stage flowers were collected and their anthers were stored at 22°C for 24 h. Flower samples for pollen viability, germination and production tests were taken at the middle of the flowering period.

The pollen viability level was determined with TTC (Norton 1966) and IKI tests (Eti 1991), 2,3,5-Triphenyl Tetrazolium Chloride solution was used in TTC test. One drop of this solution was placed on slide and pollens were spread by brush on the slide and a cover slip was placed on it. Counting was made after TTC application and it was divided into three groups based on staining density. Dark red stained pollens were referred as viable, light red as semi-viable, and unstained as non-viable (Eti 1991, Stosser 1984). Pollen grains were counted to determine viability after a couple of minutes in the IKI medium. Dark brown pollens were referred as viable, yellowish as semi-viable, and unstained as non-viable. To determine viability, about three hundred pollen grains of each replicate from four different areas were counted under a light microscope.

Pollen germination capability was determined by the "Hanging Drop" method in 0, 5, 10, 15, 20 and 25% sucrose and 0.03, 0.05, 0.1 and 0.2% boric acid media. The pollens were incubated at 22° C for 24 h under dark conditions.

Six slides and plates were scanned for each pollen source and each of these was taken as a replicate. In the germination tests, 100 grains from each replication in six microscopic areas were counted randomly. All observations of slides and plates were carried out at x 100 magnification using a light microscope.

The amount of pollen production per anther and per flower and the morphological homogeneity level of pollen were determined using the "Haemocytometric method" (Eti 1990).

The experiment was carried out according to randomized design and the values were evaluated by the Duncan's Multiple Range Test. The values for viability, germination and morphological homogeneity of pollens were subjected to arcsin square-root transformation before statistical analysis (Dowdy and Wearden 1983).

Results and Discussion

The results of TTC and IKI tests for pollen viability are shown in Table 1. The rate of pollen viability was found to be not significantly different in TTC test. In the TTC test, the highest percentage of viable pollen grains was found to be 66.93% in 25-Uz-61 and the lowest was found to be 53.34% in 25-Uz-43. The percentage of semi-viable pollen grains varied between 17.66% (25-Uz-43) and 21.19%) (25-Uz-39). The lowest percentage of non-viable pollen grains was found to be 23.53% in 25-Uz-34 and the highest was found 27.65% in 25-Uz-43 types.

Viability rates obtained by IKI test were generally close to those of TTC test. Statistically significant differences were among the observed percentages of viable and non-viable pollens in IKI test. In the IKI test the highest viable pollens was found in 25-Uz-61 (86.79%) and the lowest in 25-Uz-43 (73.02%) (Table 1).

The choice of the proper method for determining pollen viability is of great importance, both for assessing varietal composition and in breeding work in fruit cultivars. Variation in pollen quality between cultivars within a fruit species is quite common (Stosser *et al.* 1996). There are three types of pollen analysis: descriptive examinations, viability assays and physiological tests (Galletta 1983). The standard tests of viability involve *in vitro* and *in vivo* pollen germination, as well as direct estimation of the viability in ungerminate pollen grains using various chemical tests

(Stanley and Liskens 1974). Many stain tests have been used, such as aceto carmin, propion carmin, anilin blue, Alexander's stain, IKI, FDA (fluorescein diacetate), DTB (2,5-diphenyl tetrazolium bromide) and TTC to determined the pollen viability of fruit species and other plants (Oberle and Watson 1953; Werner ans Chang 1981; Widlechner *et al.* 1983; Pearson and Harney 1984; Lee *et al.* 1985, Eti and Stosser 1988, Parfitt and Ganeshan 1989, Garcia *et al.* 1990, Eti 1991, Bolat and Pirlak 1999). Stain tests have advantages as indicators of pollen viability because they are faster and easier than pollen germination. Since different results were obtained in these studies, a standard staining concentration and method could not be developed. In other words, suitable method and stain application were different for different fruit species and cultivars. Results of this study indicated that TTC and IKI stain tests could be used successfully in Cornelian cherry for which no such study has been reported before for pollen viability test.

Table 1. Pollen viability rates in different Cornelian cherry determined by TTC and IKI tests (%).

Types	TTC			IKI	
	Viable[1]	Semi-viable[1]	Non-viable1	Viable[1]	Non-viable[1]
25-Uz-34	66.2 ± 2.7	20.3 ± 1.2	13.5 ± 0.8	83.7 ± 2.8 ab	16.3 ± 1.0 bc
25-Uz-39	58.1 ± 3.5	2.1 ± 1.9	20.7 ± 1.5	82.3 ± 4.7 ab	17.7 ± 1.3 bc
25-Uz-43	54.7 ± 2.9	17.7 ± 1.6	27.6 ± 1.4	73.0 ± 3.1 c	27.0 ± 1.8 a
25-Uz-53	53.3 ± 4.2	20.4 ± 0.9	26.3 ± 1.8	77.1 ± 3.7 bc	22.9 ± 1.4 b
25-Uz-61	66.9 ± 3.0	18.1 ± 1.1	15.0 ± 1.0	86.8 ± 4.9 a	13.2 ± 1.6 c

[1]Mean ± standard error. Six replicates were used for each treatment.
Values followed by similar letter(s) do not differ significantly at 5% level.

The germination percentages of pollen grains of the Cornelian cherry are given in Table 2 and 3. It was revealed that the effects of sucrose and boric acid concentration on pollen germination were statistically significant. Increasing sucrose concentration increased germination rate up to a level (15-20%) and decreased after that point. The highest percentages of pollen germination were observed in 15% sucrose concentration in case of 25-Uz-34, 25-Uz-39 and 25-Uz-61 and 20% sucrose concentration in case of 25-Uz-43 and 25 Uz-53. The lowest percentages were observed in 0% (control) concentration in all types. It was found that the most suitable medium for pollen germination under *in vitro* conditions was 15 and 20% sucrose in some stone fruits (Werner and Chang 1981, Parfitt and Ganeshan 1984, Eti 1991).

Table 2. The percentages of pollen germination in "Hanging Drop" method at different sucrose concentrations of some Cornelian cherry .

Concentratios	Types				
(%)	25-Uz-34[1]	25-Uz-39[1]	25-Uz-43[1]	25-Uz-53[1]	25-Uz-61[1]
0	13.9 ± 0.7 d	11.5 ± 0.9 b	12.2 ± 0.9 c	11.7 ± 1.0 c	16.7 ± 0.7 c
5	21.5 ± 1.3 c	19.6 ± 1.7 ab	15.4 ± 0.8b c	17.8 ± 0.9 b	21.8 ± 1.4 b
10	28.7 ± 0.8 b	32.8 ± 2.3 a	23.1 ± 1.3 b	23.5 ± 1.7 ab	30.0 ± 2.6 ab
15	43.9 ± 3.4 a	37.3 ± 2.3 a	31.2 ± 2.6 ab	34.6 ± 2.8 a	39.0 ± 3.0 a
20	42.0 ± 3.0 a	29.0 ± 2.6 a	38.7 ± 2.2 a	37.8 ± 3.3 a	31.5 ± 2.9 ab
25	30.6 ± 3.1 b	19.7 ± 1.1 ab	22.4 ± 1.5 b	23.9 ± 2.0 b	30.2 ± 1.6 ab

[1]Mean ± standard error. Six replicates were used for each treatment.
Values followed by similar letter(s) do not differ significantly at 5% level.

The highest pollen germination rate in "Hanging Drop" method was obtained at the lowest boric acid concentration (0.03%), except for 25-Uz-39, and pollen germination rate decreased with increasing boric acid concentration (Table 3). The highest pollen germination was found to be 24.99% in 25-Uz-61, the lowest pollen germination was observed in 0.2% boric acid concentration in 25-Uz-43 (6.7%). These results are in conformity with other studies (Eti *et al.* 1990, Eti 1991, 1996). Ferrari and Wallace (1975) observed that pollen germination rate decreased when boric acid concentrations were higher than 0.08% in *Brassica* cultivars.

Table 3. The percentages of pollen germination in "Hanging Drop" method at different boric acid concentrations of some Cornelian cherry.

Concentratios	Types				
(%)	25-Uz-34[1]	25-Uz-39[1]	25-Uz-43[1]	25-Uz-53[1]	25-Uz-61[1]
0.03	17.9 ± 1.1 a	21.1 ± 0.9 a	14.3 ± 1.1	22.5 ± 1.9 a	25.0 ± 2.0 a
0.05	15.4 ± 1.0 ab	21.4 ± 1.6 a	12.1 ± 1.0	17.6 ± 1.8 ab	20.5 ± 2.1 ab
0.1	10.3 ± 1.1 b	15.5 ± 1.1 ab	9.6 ± 0.6	16.3 ± 1.9 ab	17.4 ± 1.7 ab
0.2	7.2 ± 0.8 c	11.4 ± 0.7 b	6.7 ± 0.9	10.9 ± 0.8 b	13.8 ± 1.1 b
P	**	*	N.S.	*	**

[1]Mean ± standard error. Six replicates were used for each treatment.
Values followed by similar letter(s) do not differ significantly at 5% level.
N.S. and *,** indicate non-significant and significant at 5% and 1% level respectively.

The *in vitro* pollen germination is affected by plant species from which the pollen was collected, time of collection, the season, mode of collection and conditions of its storage (Stanley and Liskens 1974). In addition to these factors, *in vitro* pollen germination is also influenced by the density of the pollen sown, the composition of germination medium, pH value, etc. (Moore and Janick 1983).

Table 4. Pollen production and morphological homogeneity values of some Cornelian cherry.

Types	Anther/Flower (No.)	Pollen/Flower (No.)	Pollen/Anther (No.)	Normal Pollen[1] (No.)
25-Uz-34	3.925	9541	2430 ab	96.0 ± 3.5
25-Uz-39	3.975	7082	1782 b	93.4 ± 2.9
25-Uz-43	3.975	10084	2537 ab	95.2 ± 3.1
25-Uz-53	3.950	13500	3418 a	92.4 ± 4.4
25-Uz-61	3.975	10959	2757 ab	92.5 ± 3.9
P	N.S.	N.S.	*	N.S.

[1]Mean ± standard error. Six replicates were used for each treatment.
Values followed by similar letter(s) do not differ significantly at 5% level.
N.S. and * indicate non-significant and significant at 5% level respectively.

Average anther numbers of investigated Cornelian cherry were found to be within 3.925 and 3.975. The significance of types was not important (Table 4). There were no statistically significant differences in terms of pollen numbers among the highest and lowest values, 13500 and 7082.5 respectively. There were significant differences as regards to the number of pollens in an anther among the types. The highest number of pollen per anther was found in 25-Uz-53 (3418) and the lowest in 25-Uz-39 (1782). Healthy development of pollen grains and ability of vigour and

germination are important for resulting in fertilization. These properties are called as pollen quality criterion, whereas pollen quantity in flowers should be of high values (Eti 1996).

Besides the amount of pollen production in the flowers of a cultivar, the rate of production of morphologically normal pollen grains is also important (Derin and Eti 2001). The highest morphological homogeneity of pollens was observed in 25-Uz-34 (96%) and the lowest in 25-Uz-53 (92.4%). The values of morphological homogeneity were generally high in investigated Cornelian cherry (Table 4). Eti (1991) reported that the values of morphological homogeneity were between 51.8-100% in different fruit species and cultivars. The highest values of morphological homogeneity can be evaluated as a good property in terms of fertilization biology. Ulkumen (1973) and Dokuzoguz (1964) reported that there is a relationship between the ratio of pollen germination and morphological structure of pollen grains and the pollen germination rate is low in the pollen grain not having morphological homogeneity.

References

Anonymous. 2001. Turkiye Istatistik Yilligi 2000. Devlet Istatistik Enstitusu, Yaym No. 2466, Ankara-Turkey.

Bolat, I. and L. Pirlak. 1999. An investigation on pollen viability, germination and tube growth in some stone fruits. Tr. J. Agr. and Forestry **23**: 383-388.

Derin, K. and S. Eti. 2001. Determination of pollen quality, quantity and effect of cross-pollination on the fruit set and quantity in the pomegranate. Tr. J. Agr. and Forestry **25**: 169-173.

Dokuzoğuz, M. 1964. Bazi önemli armut cesitlerinin dollenme biyolojisi uzerinde arastirmalar. Ege. Üniv. Ziraat Fak. Der. **1**: 64-84. (In Turkish with English Summary).

Dowdy, S. and S. Wearden. 1983. Statistics of Research. John Wiley & Sons Inc., New York.

Eti, S. and R. Stösser. 1988. Fructbarkeit der mandarinensorte 'Clementine' (*Citrus reticulata* Blanco.), I. Polenqualitat and Pollenschauchwachstum. Gartenbauwissenchaft **53**: 160-166.

Eti, S. 1990. Cicek tozu miktarmi belirlemede kullanilan pratik bir yontem. Cukurova Univ. Ziraat Fak. Der. **5**(4): 49-58. (In Turkish with English summary).

Eit, S., N. Kaska, S. Kurnaz and M. Kilavuz. 1990. Bazi yerli yenidünya (*Eriobotrya japonica* Lindl.) cesitlerinde cicek tozu uretim miktari, canhhk duzeyi ve cimlenme yetenegi ile meyve tutumu arasmdaki iliskiler. Doga Turk Tarim ve Orm. Der. **14**: 421-431. (In Turkish with English summary).

Eti, S. 1991. Bazi meyve tur ve cesitlerinde degisik *in vitro* testler yardimiyala cicek tozu canhhk ve cimlenme yeteneklerinin belirlenmesi. Cukurov Univ. Ziraat Fak. Der. **6**: 69-81. (In Turkish with English summary).

Eti, S. 1996. Yabanci kokenli bazi armut cesitlerinin dollenme biyolojileri uzerinde arastirmalar. BAHCE **25**: 11-19.

Ferrari, T.E. and D.N. Wallace. 1975. Germination of *Brassica* pollen and expression incompatibility *in vitro*. Euphytica **24**: 757-765.

Galletta, G.J. 1983. Pollen and seed management. *In:* Methods in Fruit Breeding. (Moore, J.N. and Janick, J. Eds.). pp. 23-47. Purdue Univ. Press. West Lafayette, Indiana.

Garcia, J.E., J. Egea, L. Egez and T., Berenguer. 1990. The floral biologie of certain apricot cultivars in Murcia. Hort. Abst. **60**: 9607.

Guleryuz, M. and L. Pirlak. 1996. Turkiye'de kizilcik (*Cornus mas* L.) Yetistiriciligi. Derim, **13**: 129-136. (In Turkish with English summary).

Lee, C.W., J.C. Thomas and S.L. Buchmann. 1985. Factors affecting in vitro pollen germination and storage of jojoba pollen. J. Amer. Soc. Hort. Sci. **110**: 671-676.

Moore, J.N. and J. Janick. 1983. Methods in fruit breeding. Purdue Univ. Press. West Lafayette, Indiana.

Norton, J.D. 1966. Testing of plum pollen viability with Tetrazolium salts. Proc. Amer. Soc. Hort. Sci. **89**: 132-134.

Oberle, G.D. and R. Watson. 1953. The use of 2,3,5-triphenyl tetrazolium chloride in viability tests of fruit pollens. Proc. Amer. Soc. Hort. Sci. **61**: 299-303.

Parfitt, D.E. and S. Ganeshan. 1989. Comparison of procedures for estimating viability of *Prunus* pollen. Hort. Sci. **19**: 69-70.

Pearson, H.M. and P.M. Harney. 1984. Pollen viability in rose. Hort. Sci. **19**: 710-711.

Pirlak, L. 1993. Uzundere, tortum ve Oltu Ilcelerinde Dogal Olarak Yetisen Kizilciklarm (*Cornus mass* L.) Seleksiyon Yoluyla Islahi Uzerinde Birarastirma. Unpublished Ph.D. thesis. (In Turkish with English summary).

Stanley, R.G. and H.F. Linskens. 1974. Pollen: Biology, biochemistry and management. Springer-Verlag, Berlin, Heidelberg, New York.

Stosser, R. 1984. Untersuchungen Uber die Befruchtungsbiologie and Pollen Production Innerhalb der Gruppe *Prunus domestica*. Erwerbobstbau **26**: 110-115.

Stosser, R., W, hartmann and S.f. Anvari. 1996. General aspects of pollination and fertilization of pome and stone fruit. Acta Horticulturae **423**: 15-22.

Ulkumen, L. 1973. Bag-Bahce Ziraati, Ataturk Univ. Yay. No. **275**, Ziraat Fak. Yay. No. **128**, Ders Kit. Ser,. No. **22**, Erzurum. p. 415.

Werner, D.J. and S. Chang. 1981. Stain testing viability in stored peach pollen. Hort. Sci. **16**: 522-523.

Widlechner, M.P., H. Pellett, P.D. Ascher and S.C. Fuhrman. 1983. *In vivo* pollen germination and vital staining in deciduous azaleas. Hort. Sci. **18**: 86-88.

(Manuscript received on 8 January, 2005; revised on 12 May, 2005)